

Peer Review File

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Reviewer A

It is an interesting manuscript evaluating function of extracellular RNAs in lung cancer. However, major revision is required for current paper. Please change the title and make it more elaborate and currently, it appears to be a review paper not a research paper. The first paragraph of introduction needs edition and please discuss about chemoresistance, radioresistance and challenges in treatment of lung cancer.

highly stable and highly resistant. One "highly" is enough.

Description of circRNAs in lung cancer needs more elaboration in introduction.

The description of work at the end of introduction is not enough.

A: We changed it accordingly and added a description.

Reviewer B

1. What was the criteria for selection of normal cohort? Which clinical parameters were taken into consideration?

A: The control group selected blood samples from healthy volunteers. Blood samples were collected from patients with Stage I-III resectable non-small cell lung cancer.

2. Figure 2 is not visible.

A: We modified Figure 2

3. Since, figure 1 and figure 2 only describe the abundance of reads, these two figures can be merged.

A: Thanks for the reviewer's suggestion, Figure 1 and Figure 2 can be merged, but for the sake of typesetting, we choose to describe them separately.

4. A volcano plot or a heatmap must be shown for differentially expressed genes.

A: We add the volcano plot of different expressed genes between NSCLC and normal control in figure 3.

5. What was the cut-off for differential expression?

A: fold change >2, P<0.05

6. Line number 241-242, what was the criteria for selection of circ_0000722 and circ_0006156?

A: Select according to P-value ranking, and select the lowest value.

7. It is not clear what is meant by circFARSA in figure 6 legend? There is no mention of this in the entire manuscript.

A: Thanks for the reviewer's careful guidance. This is a clerical error. It should be circRNAs, which has been modified in the illustration in Figure 6.

8. Authors need to mention how were the targets of miRNAs predicted and what was the selected cut-off for targets shown in figure 6B. Also, does the color code in the figure represent anything, please mention this in figure legend?

A: TargetScanHuman subdatabase in TargetScan database was used to predict the targets of miRNAs. The colors don't mean anything, just different sets of genes

9. Line numbers 276-280, a definite inference cannot be drawn based on this upregulation. Authors need to modify this statement.

A: Thanks for the reviewer's reminding, we have made corresponding modifications.

10. Has the sequencing data been submitted to any public databases? No information has been provided regarding this. Also, no supporting data or list of genes has been provided for the differentially expressed transcripts.

A: The RNA-seq datasets are submitted and are available at NCBI project PRJNA971084 (<https://www.ncbi.nlm.nih.gov/>, accessed on 16 May 2023). We all added description in the section of "Summary of the sequencing data".

11. How would authors justify the source of circRNAs in the plasma of NSCLC patients?

A: Thank you for your valuable suggestion, we only profile the different expressed plasma circRNAs between NSCLC patient and controls in this present study, and we will study the source of plasma circRNAs in the future.

12. There is typographical error in the entire manuscript and figure legends pertaining to the nomenclature of miRNAs, "has" should be replaced with "hsa".

A: Thanks to the reviewer's reminding, we made the modification.

13. Authors should mention sequencing read-depth used in this study.

A: we showed the sequencing read-depth in the first paragraph of the result section.

Reviewer C

The paper titled “Extracellular RNA profiles in non-small cell lung cancer plasma” is interesting. In this study, an exRNA-sequencing strategy was used to identify the expression of NSCLC-specific transcription factors in clinical plasma samples, and hsa_circ_0000722 and hsa-miR-324-5p were identified as potential biomarkers in NSCLC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The identifications in the figure are inconsistent with those in the figure legends and result, for example, a and b are used in Figures, but A and B are used in the figure legends and result. Uniform identification is recommended.

A: We have modified and uniformed the figure legend and result.

2) The bioinformatics analysis in this study is too simple. It is recommended to conduct WGCNA analysis on the data to determine the key modules, which may be more meaningful.

A: Thanks for the reviewer's suggestion. We will further increase relevant research and analysis in the follow-up large-sample verification.

3) Many words in Figure 2 are obscured and cannot be seen clearly. Please change the words or background color. Also, ABCD is not displayed in Figure 2 and 3, please add on.

A: We modified these figures.

4) What are the exRNAs function in cancer drug resistance? It is recommended to add relevant content.

A: We changed it accordingly and added a description.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “The extracellular RNA and drug resistance in cancer: a narrative review, Transl Lung Cancer Res, PMID: 35529788”. It is recommended to quote the articles.

A: We quoted this article in the introduction part.

6) It is suggested to add further experimental studies to clarify the specific role and molecular mechanism of key molecules in this study.

A: Thanks for the reviewer's suggestion. We will further increase relevant research and analysis in the follow-up large-sample verification.

7) How can the results of this study help to develop therapeutic strategies against NSCLC? It is recommended to add relevant content.

A: We added relevant contents.

Reviewer D

1. Reporting Checklist

No related information was found in the main text about these items, please double check. If it is not applicable, please fill with N/A.

Design

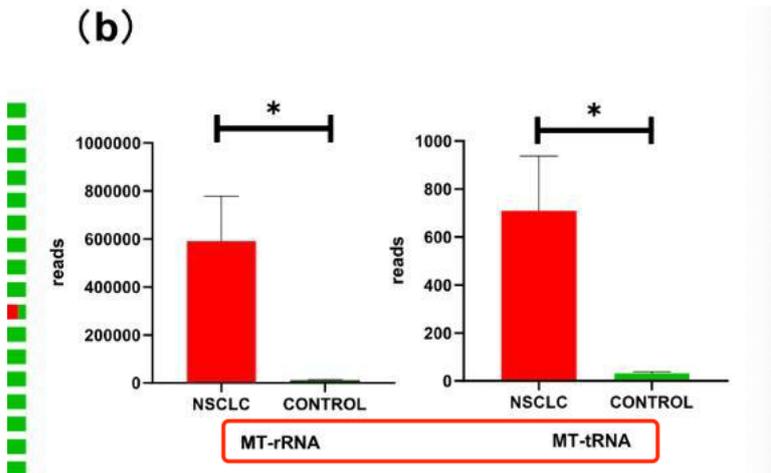
Study protocol	Yes (indicate where provided: section/paragraph)	n/a
For clinical trials, provide the trial registration number OR cite DOI in manuscript.	The first paragraph of the "Materials and Methods" section (line 76)	
Experimental study design (statistics details)	Yes (indicate where provided: section/paragraph)	n/a
State whether and how the following have been done, or if they were not carried out.		
Sample size determination		✓
Randomisation	The first paragraph of the "Materials and Methods" section	
Blinding	The first paragraph of the "Materials and Methods" section	
Inclusion/exclusion criteria	The first paragraph of the "Materials and Methods" section	

Response: We have added some related information in the checklist and revised manuscript.

2. Figure 1

Please check which is correct, mtmt or MT?

- (b) The changes in the total reads of the mtmt-rRNA, mtmt-tRNA and protein coding



Reply: We are so sorry for the negligence. "mt" is correct. We have revised this mistake in the revised manuscript and revised figure 1

3. Figure 3

Please explain FC in the legend.

Reply: FC, fold change. We have added it in the figure legend

4. References/Citations

a) Reference 28 was not cited in the main text, please revise. Please note that it should be cited between 27 and 29.

Reply: Thank you very much for correctly pointing out this mistake. We have added the citation in the revised manuscript.

b) Please double-check if more studies should be cited as you mentioned “studies”. OR use “study” rather than “studies”.

105 of cancers (25,26). ~~Recent studies have shown that circRNAs may be involved in the~~
106 ~~tumorigenesis and development of lung cancer, and relevant studies on circRNAs are~~
107 ~~of great significance for the diagnosis and treatment of lung cancer~~ . However, the

Reply: We have used “study” instead of “studies”.